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What are the advantages of cortical *versus* nuclear organization of homologous neuronal circuits? My earlier proposal that the avian brain contains cells and circuitry which are nearly identical to those in the mammalian cortex, but disposed as nuclei rather than layers with interlaminar reciprocal connections, is strongly supported by the recent report by Dugas-Ford *et al.* [1]. Recently, however, Wang *et al.* [11] directly demonstrated the existence of radial columnar organization within the auditory region of the telencephalon, with recurrent loops strikingly similar to that long recognized as a characteristic of the mammalian sensory cortex (Figure 2A,B). They suggested that this radially organized columnar processing unit evolved at least 250 million years ago. Recent studies of the zebra finch brain by Woolley *et al.* [12], Kim and Doupe [13] and others have started to clarify the role of each of the constituent populations of this radial column in the auditory 'cortex' and provide insight into the presumably ancient mechanisms of computational processing common to different classes of vertebrates, including mammals. The simple dichotomy of laminar *versus* nuclear organization in mammals vs. birds was a useful heuristic model to understand the evolution of cortex, and now may lead to ever more intriguing questions about evolution of the refined microcircuitry so characteristic of mammalian cortex.

We are now confronted with the challenges of specifying mechanisms underlying cell typology and the molecular regulation involved in building highly conserved, small specialized microcircuits. A still greater problem of how macroarchitectural components such as regionally distinct laminated cortical areas are formed in both evolution and embryogenesis remains to be addressed.

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Development: Do Mouse Embryos Play Dice?

Cells of early mouse embryo were considered for a long time to acquire cell fate at random. Recent analyses argue against this simple model.

Magdalena Zernicka-Goetz

The question of how cells of early mammalian embryos first become different from each other — either entering the path towards pluripotency or the path towards differentiation — has been attracting substantial interest for many years. Undeniably, as the mouse embryo is a model system to understand development of the human embryo, it raises two of the most important questions about our own development: 'How do cells start to develop their unique identity to establish the shape and pattern of the body?' and 'when does this all begin to happen?' Understanding the answers will help us in many ways, from finding the best way to generate stem cells and guide their differentiation process, to how to select cells for genotyping embryos fertilized and developing *in vitro*.
In the embryos of most model organisms, the earliest differences between cells arise as a result of polarisation of the egg, which causes regulatory molecules, 'determinants', to become asymmetrically localised. But the way mammalian embryos work is most likely different — it is more 'democratic'. Cells of mammalian embryos have not been observed, thus far, to inherit maternally provided instructions — they need to 'make up their minds' about what to do and then influence the majority decision. So how do pattern and form begin to develop in the mammalian embryo? In this issue of *Current Biology*, Kevin Eggan and colleagues [1] shed light on these questions by using a novel cell labelling technique to follow the developmental contributions made by individual early mouse embryo cells, called at this stage, blastomeres.

Mouse embryo development starts with a series of precise cleavage divisions that after four days result in a blastocyst with three cell types found in distinct layers: 'outside' cells of the trophectoderm and two layers of 'inside' cells, the inner cell mass, that form either the epiblast or the primitive endoderm (Figure 1A). Epiblast cells

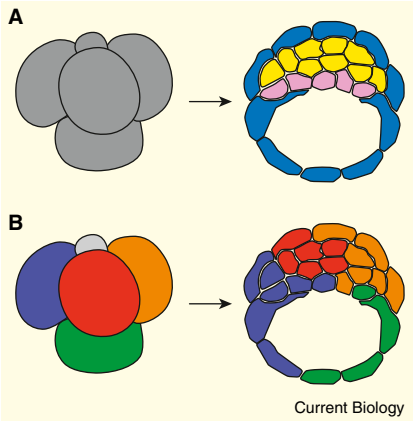

Figure 1. Contributions of rainbow-labelled clones in mouse blastocysts.

Diagrammatic sections of 4-cell and blastocyst stage mouse embryo to illustrate: (A) three distinct lineages of the blastocyst: epiblast (yellow), primitive endoderm (pink) and trophectoderm (blue). (B) Labeling each 4-cell blastomere by the rainbow technique and the distribution of the resulting four clones of cells at the blastocyst stage. Note the prominent contribution made by the green clone to the outer trophectoderm layer and by the red clone to the inner cell mass.

are pluripotent and give rise to cells of the future embryo while trophectoderm and primitive endoderm are extra-embryonic tissues with signalling and supportive roles for the epiblast when it starts to construct the body. The question is how does a ball of apparently identical cells become organised into these three crucial cell layers? Clues to the answer came first from observing how the cleavage divisions partition the egg into the component parts of the blastocyst. The first cell division is typically meridional, along the 'animal-vegetal' axis that is marked by the polar body extruded at the animal pole of the egg during meiosis [2,3]. The second cleavage divisions are either meridional or equatorial and generate 4-cell embryos, in which each cell looks like its neighbour. After the next cleavage division, each cell becomes polarised along the apical-basal axis and undertakes either a symmetric or an asymmetric division. If a cell divides symmetrically, it generates two outside polarised daughter cells. If it divides asymmetrically, it generates one outside, polarised daughter cell and one non-polar, inside daughter cell. Outside cells adopt a trophectoderm fate while the inside cells contribute to either the epiblast or the primitive endoderm depending largely on which of the three waves of asymmetric division generated them [4,5]. Thus, asymmetric divisions mark the first visible fate-determining events as the ball of cells is fashioned into the blastocyst with its three cell types.

For a long time it has been believed that cells are identical within this ball at least until they take up distinct inside and outside positions, but is this true? A number of studies [2,3,6–9] over the last 10 years involving a variety of markers such as oil-drops, dyes, fluorescent beads and proteins, together has challenged this view. These studies indicated that cells start to develop their identity earlier than expected, before taking inside or outside positions and, importantly, that these differences between cells are significant, as they bias cell fate [2,3,6–9]. This view has not been universally held, however, because differences between the cells were not detected by other groups and so the view that cell fate choice is random was maintained [10–12]. It was also proposed that the bias in cell fate might simply result from the shape of the zona pellucida surrounding the embryo until implantation [13], an interpretation that has itself been questioned [14]. The random (stochastic) view of development became popular as it seemed to fit more comfortably with the experiments showing that early embryo cells are plastic and can adopt different fates upon transplantation.

The new contribution from Kevin Eggan's group [1] now further challenges the view that early mouse development is stochastic. It clarifies earlier lineage studies by borrowing the 'brainbow' technique originally developed to mark clones of neuronal cells in multiple colours. This technique uses a transgene with tandem arrays of different fluorescent protein genes separated by loxP sites. In the absence of Cre-induced recombination, the transgene expresses the fluorescent protein gene that is immediately adjacent to its promoter. But remarkable technicolour embryos are produced by driving Cre expression at specific stages from a tamoxifen responsive promoter, which leads to one of several mutually exclusive excision events that position a different fluorescent protein gene adjacent to the promoter and generate multiple clones expressing fluorescent proteins of different colours.

Using this approach, Eggan and colleagues [1] induce recombination in the cleavage stage embryo to determine the contribution of individual blastomeres to embryonic and extra-embryonic lineages at the blastocyst and post-implantation stages. They find that when recombination is induced as early as the 4-cell stage, individual blastomeres give rise to either the inner cell mass or to the trophectoderm lineages in a large group of embryos (Figure 1A,B). The observed bias in cell fate is clear and significant and the authors conclude that it cannot be accounted for simply by the shape of the zona pellucida. Excitingly, at post-implantation stages, the clones derived from individual blastomeres are also found in either the epiblast or extra-embryonic tissues, indicating that the early bias has relevance to subsequent development. These results led the authors to conclude that cells in the early mouse embryo do differ in their cell fate choices and that "it is unlikely that bias we observed in this data set could be explained by chance" [1].

These elegant experiments with rainbow-mice are extremely important because they clarify the long-standing debate about inequalities in mouse embryo cells starting already by the 4-cell stage. The first evidence for such inequalities came from the discovery that one 4-cell blastomere enters the differentiation pathway ahead of its sister and cousin cells — it generates significantly more cells that differentiate into trophectoderm than pluripotent epiblast tissue [15]. With this knowledge, it was then possible to build chimeric embryos consisting entirely of 4-cell blastomeres that preferentially generate trophectoderm. These chimeras were unable to develop to birth [15], most likely due to an inability to generate a critical number of pluripotent cells [16], while chimeras of blastomeres that contribute to both inner cell mass and trophectoderm developed normally. In a separate study [17], it was found that the direction of axial rotation of the foetus is also affected by the choice of 4-cell blastomeres used to build chimeras. Clues to the molecular mechanism underlying these early inequalities came from studies that link a specific epigenetic modification, histone H3R26 methylation, to cell fate [18]. This histone mark appears to be

the earliest identifier of the choice to develop pluripotency and indeed changing H3R26 methylation status, changes the expression of pluripotency genes and consequently cell fate. Subsequent time-lapse studies of unperturbed embryos revealed that the progeny of the 4-cell blastomere shown to have lowest H3R26 methylation divides symmetrically, thus explaining its biased contribution to trophectoderm [19]. Finally and most recently, 4-cell embryo blastomeres were found to differ in kinetics of the transcription factor Oct4 in a way that further explains their differing fates [20]. Collectively, these studies strongly suggest that at least one cell of the 4-cell embryo differs from the others: this cell's descendants initiate differentiation earlier than those of the other three cells.

Perhaps it is now time to accept that cells in the mouse embryo do not acquire identity in an entirely random (stochastic) manner and that differences between cells can arise as early as the 4-cell stage. It is clear that cells are still developmentally flexible at this point, and even later, but left undisturbed they seem to have 'preferred' paths. An attractive hypothesis that might reconcile many older and newer findings would be that the mouse embryo cells are influenced by where they come from and the circumstances of their parents — they have a memory of their developmental history — but remain flexible enough to adapt to life's new circumstances. This hypothesis illustrates the beauty of this developmental system. Numerous approaches have been devised to show that embryo cells taken from their native environment can flourish when transplanted to new sites; indeed cell properties change upon isolation. What we now need to find out is how these early cells begin to acquire their differences in normal development at a time that precedes the impact of inside versus outside positioning. This will be of great help in understanding the first molecular steps on a path to differentiation on one hand, and on a path to pluripotency on the other. What might the mechanism be? Does it relate to polarisation of the egg or of the zygote? If so, is it influenced by the dramatic asymmetry of the meiotic divisions, asymmetry introduced by sperm entry at fertilisation, or by the behaviour of the male and female pronuclei in the first cell division? Whatever the factor or factors, they are likely to be quite subtle and so their identification presents a challenge, but an exciting one.

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Ras GTPases: Codon Bias Holds KRas Down but Not Out

Rare codons selectively limit the accumulation of Ras family member proteins with important consequences for Ras pathway activation and tumorigenesis.

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Orchestration of dynamic cell-biological responses to regulatory cues almost invariably mobilizes Ras family small GTPases for signal distribution and integration. This, together with the recognition that *KRAS* is the most frequently mutationally activated human oncogene, has inspired intense efforts to unravel the mechanistic details of Ras-dependent signal transduction. In humans, three genes (*HRAS*, *KRAS*, and *NRAS)* encode four nearly identical